Karyotype of a Ranid Frog, Platymantis pelewensis, from Belau, Micronesia, with Comments on Its Systematic Implications

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Karyotype of a Ranid Frog, *Platymantis pelewensis*, from Belau, Micronesia, with Comments on Its Systematic Implications[1]

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ABSTRACT: The karyotype of *Platymantis pelewensis* Peters, 1867, the only native, endemic amphibian in Belau, extremely isolated from other congeners, consisted of $2n = 22$ homologous chromosomes largely forming a graded series. Of these, chromosomes of pairs 1, 5, 6, 8, 10, and 11 were metacentric; the remainder were submetacentric. Secondary constrictions were evident on shorter arms of pair 7. This karyotype is nearly identical with that of *P. papuensis* Meyer from New Guinea, but is distinct from two Philippine congeners hitherto karyotyped both in chromosome number and morphology. This suggests that the ancestral form of *P. pelewensis* dispersed from New Guinea or other Melanesian islands.

THE RANID GENUS *Platymantis* Günther consists of 37 species distributed in the Philippines, Belau (Pelew or Palau), Melanesia (including New Guinea), and western Polynesia (Frost 1985, but see Zhao and Adler [1993] for the status of Chinese species assigned to the genus). The range of the genus is characteristic of that of most other Australasian frog genera in that it spreads onto various oceanic islands (see above) (Inger 1954, Darlington 1957, Gorham 1965). It is generally supposed that species currently isolated on such islands represent past multiple overseas dispersals and subsequent speciations (Brown 1965, Tyler 1979, Gibbons 1985). Gibbons (1985) further surmised that the highly specialized mode of reproduction in the genus, involving the direct development of embryos (Atoda 1949, Brown and Alcala 1982a), has been especially advantageous in the process of its colonization of oceanic islands.

However, there has been little information regarding the process of divergence among *Platymantis* species: origins and relationships especially of a few, isolated species remain unknown. Of these, *P. pelewensis* Peters, 1867, the only native and endemic amphibian species of Belau, western Micronesia, is one of the most poorly studied species despite its great biogeographical interest. We have examined the karyotype of *P. pelewensis* for the first time and discuss its phylogenetic and biogeographical relationships with other congeneric species from the cytotaxonomic standpoint.

MATERIALS AND METHODS

Two males were collected from Ngergoi Island, ca. 27 km SW of Coror Island, and were taken live to the laboratory, where they were injected with colchicine solution (0.1 mg/ml) in their femoral muscles at 0.1 ml/g body weight. They were sacrificed about 10 hr after the injection. Bone marrow, extracted from the femurs, was subjected to hypotonic treatment in KCl solution (0.05 mol/liter) for 40 min and then rinsed and fixed in Carnois's solution (glacial acetic acid: methanol, 1 : 3). The cell slides, prepared by the air-dry method, were soaked in 3% Giemsa solution for 30 min and then were investigated microscopically and photographed. The karyotype was determined on the basis of five well-spread metaphase cells for each individual.

The arm length of each chromosome was

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measured on the photoprint using an iron string. Terminology for chromosomal descriptions follows Green et al. (1980). Voucher specimens were deposited in the amphibian collection of the Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan.

RESULTS AND DISCUSSION

Both males had a karyotype consisting of $2n = 22$ homologous chromosomes largely forming a graded series. Of these, the chromosomes composing pairs 1, 5, 8, 10, and 11 exhibited arm ratios smaller than 1.70 and hence were classified as metacentric. The remainder exhibited ratios ranging from 1.79 (pair 6) to 2.44 and were classified as submetacentric. Secondary constrictions were evident on shorter arms of pair 7 (Figure 1, Table 1).

Of the 37 valid species of the genus *Platymantis*, only three, *P. hazelae* (Taylor) and *P. dorsalis* (Duméril) from the Philippines, and *P. papuensis* Meyer from New Guinea, have hitherto been karyotyped (Kuramoto 1985). The karyotype of *P. pelewensis* is distinctly differentiated from those of the two Philippine species both in the chromosome number (*P. hazelae* has 26 chromosomes and *P. dorsalis* has 20 chromosomes) and in morphology. However, it closely resembles the karyotype of *P. papuensis* ($2n = 22$), from which it differs slightly by having pair 6 submetacentric (arm ratio = 1.79) instead of metacentric (arm ratio = 1.60), and secondary constrictions in pair 7 (Table 1).

Platymantis pelewensis was first described as *P. plicifera* var. *Pelewensis* by Peters (1867), who briefly stated that it differs from *P. plicifera* Günther from the Philippines (subsequently designated a synonym of *P. corrugatus* [Duméril] by Inger [1954]) only in the lateral head coloration. However, Inger (1954), on the basis of far more detailed morphological comparisons, considered that the species is most closely related to *P. meyeri* Günther from the Philippines (later designated a synonym of *P. dorsalis* by Brown and Inger [1964]) and *P. papuensis* and *P. rubristriatus* (Barbour) from New Guinea (*P. rubristriatus* was designated a synonym of *P. papuensis* by Zweifel [1969]). Gorham (1965), although basically accepting Inger's (1954) account, considered that *P. pelewensis* is more closely related to *P. dorsalis* than to *P. papuensis* on the basis of morphological similarity. Since Inger's (1954) work, five species have been added to the genus from the Philippines, but because of their distinct morphological divergences and specializations (see Brown and Alcala [1963, 1974, 1982b] for further details), Inger's (1954) and Gorham's (1965) assumption of the closest affinity of *P. dorsalis* with *P. pelewensis* among the Philippine species appears still valid.

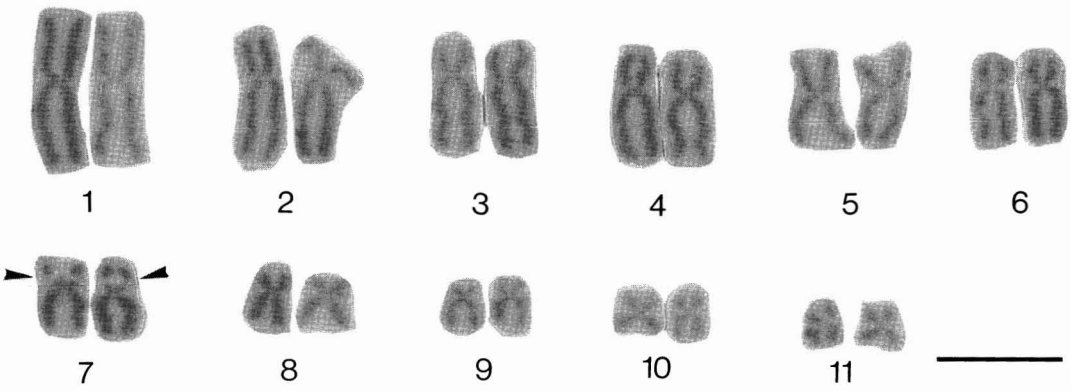

FIGURE 1. Karyotype of male *Platymantis pelewensis* from Belau. Bar equals 10 μm. Arrows indicate positions of secondary constrictions on pair 7.

TABLE 1

CHROMOSOMAL COMPARISONS OF *Platymantis pelewensis* WITH OTHER CONGENERIC SPECIES HITHERTO KARYOTYPED

	SPECIES (LOCALITY: DIPLOID NO.)											
	P. pelewensis (BELAU: 22)			*P. papuensis* (NEW GUINEA: 22)			*P. dorsalis* (PHILIPPINES: 20)			*P. hazelae* (PHILIPPINES: 26)		
PAIR NO.	RELATIVE LENGTH	ARM RATIO	TYPE	RELATIVE LENGTH	ARM RATIO	TYPE	RELATIVE LENGTH	ARM RATIO	TYPE	RELATIVE LENGTH	ARM RATIO	TYPE
1	16.1 ± 0.18	1.27 ± 0.03	m	16.0 ± 0.16	1.26 ± 0.01	m	17.4 ± 0.36	1.08 ± 0.01	m	15.6 ± 0.24	1.27 ± 0.02	m
2	12.8 ± 0.19	1.90 ± 0.03	sm	13.2 ± 0.13	1.89 ± 0.03	sm	13.0 ± 0.21	4.19 ± 0.27	st	12.9 ± 0.18	1.70 ± 0.03	sm
3	11.7 ± 0.12	2.25 ± 0.07	sm	11.6 ± 0.13	2.27 ± 0.06	sm	12.3 ± 0.13	1.27 ± 0.03	m	11.2 ± 0.26	2.17 ± 0.03	sm
4	11.3 ± 0.21	1.95 ± 0.05	sm	11.3 ± 0.14	1.90 ± 0.05	sm	11.6 ± 0.17	2.77 ± 0.10	sm	10.8 ± 0.12	1.83 ± 0.05	sm
5	10.4 ± 0.20	1.35 ± 0.02	m	10.2 ± 0.10	1.38 ± 0.02	m	10.1 ± 0.15	1.25 ± 0.03	m	9.9 ± 0.09	1.33 ± 0.02	m
6	9.6 ± 0.08	1.79 ± 0.04	sm	9.1 ± 0.06	1.60 ± 0.03	m	8.6 ± 0.14	1.12 ± 0.02	m	5.8 ± 0.12	1.33 ± 0.03	m
7	7.1 ± 0.08	2.44 ± 0.11	sm[a]	7.6 ± 0.10	1.89 ± 0.05	sm	7.8 ± 0.10	1.15 ± 0.04	m	5.7 ± 0.12	2.40 ± 0.06	sm
8	5.8 ± 0.16	1.56 ± 0.05	m	5.9 ± 0.08	1.61 ± 0.04	m	7.3 ± 0.11	1.12 ± 0.02	m	5.3 ± 0.08	1.33 ± 0.04	m
9	5.5 ± 0.13	1.96 ± 0.06	sm	5.3 ± 0.07	2.08 ± 0.04	sm	6.8 ± 0.16	1.22 ± 0.05	m	5.0 ± 0.09	2.31 ± 0.08	sm
10	5.1 ± 0.12	1.48 ± 0.08	m	5.2 ± 0.07	1.42 ± 0.03	m	5.1 ± 0.13	1.14 ± 0.02	m	4.9 ± 0.08	1.26 ± 0.03	m
11	4.5 ± 0.09	1.28 ± 0.03	m	4.5 ± 0.05	1.26 ± 0.02	m				4.7 ± 0.08	1.20 ± 0.05	m
12										4.5 ± 0.09	1.24 ± 0.04	m
13										3.8 ± 0.09	2.99 ± 0.15	sm

NOTE: Data presented as means ± SE. Abbreviations: m, sm, and st represent metacentric, submetacentric, and subtelocentric chromosomes, respectively. Data for *P. papuensis*, *P. dorsalis*, and *P. hazelae* from Kuramoto (1985).

[a] With secondary constrictions on shorter arms.

Our comparisons have revealed that *P. pelewensis* resembles *P. papuensis* much more closely than *P. dorsalis* chromosomally. Kuramoto (1985) assumed that karyotypes of *P. papuensis* and *P. dorsalis* represent two differently specialized conditions derived from the $2n = 26$ karyomorph represented by the karyotype of *P. hazelae*. He pointed out that the karyotype of *P. papuensis* could have derived merely by centric fusion and pericentric inversion, whereas that of *P. dorsalis* would have been involved in more complicated chromosomal rearrangements. If this assumption is accepted, close chromosomal similarity of *P. palawensis* with *P. papuensis* but not with *P. dorsalis* can be interpreted as indicative of the closer phylogenetic relationships of the former two species.

Tyler (1979), in discussing historical relationships of *Platymantis* species in the eastern Oriental and Pacific regions, provided a schematic figure (i.e., fig. 4 : 4) indicating that the ancestral form of *P. pelewensis* had dispersed from the Philippines, presumably on the basis of Gorham's (1965) account (see above). However, our results seem to indicate that the ancestor of *P. pelewensis* actually dispersed from New Guinea or adjacent Melanesian islands, similar to ancestors of some reptilian species in Belau (Ota et al. 1995). Further chromosomal surveys and phylogenetic analyses of *Platymantis* species are needed to verify our hypothesis.

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